#28 5-6-01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty. Docket: TOVEY=2

In re Application of:	)	Art Unit: 1646
Michael G. TOVEY	)	Examiner: J. Andres
Appln. No.: 08/853,292	)	Washington, D.C.
Filed: May 9, 1997	)	
For: STIMULATION OF HOST DEFENSE MECHANISMS AGAINST VIRAL CHALLENGES	)	

DECLARATION

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

I, the undersigned Michael G. TOVEY hereby declare and state as follows:

I am the inventor of the above-identified patent application. I hold a Ph.D. from the University of London in microbiology. I am presently, and have been for approximately ten years, Director of the Laboratory of Viral Oncology, CNRS UPR 9045 at the Institut Andre Lwoff (French National Cancer Institute). I am also INSERM (French National Institute of Health) Director of Research at CNRS. I am also professor of molecular biology at the University of Paris XI.

I am the senior author of the publication "Oromucosal Interferon Therapy: Marked Antiviral and

Antitumor Activity", J Interferon Cytokine Res 19(2):145-155 (1999), a copy of which is attached hereto. All of the experimentation disclosed and discussed in said publication was either conducted by me or under my supervision. I hereby state of my own knowledge that all of the experimental results therein are true and correct to the best of my knowledge and belief.

The experimental results, such as those shown in Figure 1, Figure 2 and Figure 4, establish that the antiviral activity of oromucosal interferon therapy is dose responsive. These results were consistent with the following statement in the above-identified patent application at page 20, lines 5-7:

> In keeping with these results the extent of the antiviral activity exerted by oromucosally administered IFN did appear to follow a classical dose-response relationship.

The dose response results discussed above were surprising to me at the time as conventional wisdom would hold that indirect immunological stimulation of a substance that is not absorbed by the organism in appreciable quantities would not be dose responsive. There have been previous reports that interferon stimulation of immune-mediated effects, which is effective in low doses, is inhibitory at higher doses. These include such immune-mediated effects as NK cell cytotoxicity (Edwards et al, "Low doses of interferon alpha result in more

effective clinical natural killer cell activation", J Clin Invest 76(6):1908-1913 (1985)), antibody production (Peters et al, "Effect of interferon-alpha on immunoglobulin synthesis by human B cells", J Immunol 137(10)3153-3157 (1986) and Weiss et al, "Effect of recombinant human interferon-alpha in vitro and in vivo on mitogen-induced lymphocyte blastogenesis in cats", Vet Immunol Immunopathol, 24(2):147-157 (1990)), and major histocompatibility antigen expression (Rosa et l, "Presence of an Abnormal β_2-Microglobulin mRNA in Daudi Cells: Induction by Interferon", Immunogenetics 17:125-131 (1983)). Abstracts or full text of the publications cited in this paragraph are attached hereto.

Accordingly, the classical dose-response relationship at dosages substantially higher than the dosage designated as the maximum dosage for stimulation of the immune system by oromucosal administration of interferon disclosed in Cummins U.S. patents 5,019,382 and 5,830,456 is unexpected and unobvious. Those of ordinary skill in the art, at the time the invention of the above-identified application was made, would have had no reasonable expectation that the use of an amount of interferon twice that of the maximum specified by Cummins, or higher, would be effective; indeed, they might have expected it to be ineffective.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

19 June 2001.
Date

Michael G. TOVEY

1: J Clin Invest 1985 Jun;75(6):1908-13 Related Articles, Books

PubMed Services

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Low doses of interferon alpha result in more effective clinical natural killer cell activation.

Edwards BS, Merritt JA, Fuhlbrigge RC, Borden EC.

To define critical parameters concerning interferon (IFN) effects upon natural killer (NK) cells in vivo, we gave cancer patients serial weekly intramuscular injections of purified lymphoblastoid IFN in six doses ranging from 10(5) to 3 X 10(7) U. Dose sequences were determined by randomly allocating patients to one of six levels in a latin square ordering scheme. NK cell stimulation, a threefold peak increase above preinjection levels of cytolysis (P = 0.022), occurred in peripheral mononuclear cells (PMC) sampled 24 h postinjection, of 3 X 10(6) U, but was not detectable at any dose in PMC sampled 7 d postinjection. No blunting occurred in NK cell responsiveness to repeated injection of IFN dosages a second time at or several weeks after study completion. At IFN doses of 3 X 10(6), 10(7), and 3 X 10(7) U, a negative correlation existed between the amount of IFN injected and the average extent of NK cell activation (r = -0.423, P less than 0.05). This contrasted with the progressively increasing response of NK cells to in vitro incubation with increasing concentration of up to 3,000 U/ml of IFN. Overnight culturing of PMC sampled before IFN injections resulted in a mean 1.9-fold increase in cytolytic activity (P = 0.0005) and a mean 53% decrease in variance (P = 0.024) between serial preinjection NK cell activity determinations. Cell separation procedures may, therefore, have resulted in NK cell inactivation, from which overnight culturing permitted recovery. We found that maximal NK cell activation at a low IFN dose, decreasing NK cell responsiveness at higher doses, and the need to culture PMC to efficiently detect NK cell boosting may account for disparities in reported effects of IFN on NK cell function.

Publication Types:
- Clinical trial
- Randomized controlled trial

PMID: 4008643 [PubMed - indexed for MEDLINE]

Effect of interferon-alpha on immunoglobulin synthesis by human B cells.

Peters M, Ambrus JL, Zheleznyak A, Walling D, Hoofnagle JH.

We have investigated the effect of human recombinant interferon-alpha (IFN-alpha) on mitogen-induced immunoglobulin (Ig) production by peripheral blood mononuclear cells from normal individuals. Low concentrations (1 to 100 IU/ml) of IFN-alpha enhanced pokeweed mitogen-stimulated Ig production. In contrast, high concentrations of IFN-alpha (10(5) IU/ml) suppressed pokeweed mitogen-induced Ig production. Irradiation of T cells did not ablate the high dose suppression, indicating that suppression was not due to a radiation-sensitive T cell. Kinetic experiments revealed that IFN-alpha needed to be added to 10 day cultures within the first 72 hr for either enhancement or suppression to be noted. Preincubation of purified B cells with IFN-alpha suppressed Ig production as completely as when unfractionated mononuclear cells were incubated with IFN-alpha. On the other hand, preincubation of T cells or monocytes with IFN-alpha had no effect on subsequent Ig production in reconstituted mononuclear cell cultures. Mitogen-induced proliferation of purified B cells was not affected by IFN-alpha at any concentration, but Ig production by purified B cells stimulated with Staphylococcus aureus Cowan I or anti-mu and B cell differentiation factors responded to IFN-alpha with low concentration enhancement and high concentration suppression. Studies of Ebstein-Barr virus-transformed B cell lines showed that IFN-alpha caused a similar effect on the CESS line as on peripheral blood B cells, with low dose enhancement and high dose suppression of Ig production. Thus one IFN-alpha effect is to modulate Ig production, and this appears to be a direct effect on B cells. Combined with the data in the accompanying paper, the effects of IFN-alpha on B cell function are similar in vivo and in vitro.

PMID: 3021846 [PubMed - indexed for MEDLINE]

1: Vet Immunol Immunopathol 1990 Feb;24(2):147-57 Related Articles, Books

Effect of recombinant human interferon-alpha in vitro and in vivo on mitogen-induced lymphocyte blastogenesis in cats.

Weiss RC, Oostrom-Ram T.

Scott-Ritchey Research Program, College of Veterinary Medicine, Auburn University, AL 36849.

The effect of recombinant human interferon-alpha (rHuIFN-alpha) in vitro and in vivo on mitogen-induced lymphocyte blastogenesis was evaluated in specific-pathogen-free cats. Pre-incubation of isolated feline peripheral blood lymphocytes (PBL) in vitro with either 10(4) or 10(3) International Units (U) of rHuIFN-alpha for 24 h significantly suppressed (P less than 0.001 and 0.01, respectively) blastogenic responses to the phytomitogens concanavalin A (Con A) and pokeweed mitogen (PWM). Lower doses of IFN (range, 10-10(-3) U/ml) neither suppressed nor enhanced mitogenesis. In the absence of phytomitogens, incubation of PBL with 10(4) - 10(2) U (P less than 0.001) or 10 U (P less than 0.05) of rHuIFN-alpha/ml resulted in a significant decrease in incorporation of [methyl-3H] thymidine into newly synthesized cellular DNA. Cultures of PBL exposed continuously for 4 days to rHuIFN-alpha doses of 10(4) U/ml or less did not demonstrate specific reductions in cell viability, indicating that the observed antiproliferative actions of IFN apparently were independent of any direct cytotoxic effects. To investigate the dose-response effects of rHuIFN-alpha in vivo on lymphocyte blastogenesis, individual groups of cats were evaluated on 3 consecutive days before and then 24 h after each cat was inoculated intramuscularly with either a high dose (10(6) U/kg), moderate dose (10(4) U/kg), or a relatively low dose (10(2) U/kg) of rHuIFN-alpha. Cats inoculated with 10(6) U of rHuIFN-alpha/kg had significantly reduced (P = 0.037) blastogenic responses to Con a at 24 h postinoculation compared to preinoculation values; mean PWM responses were also decreased, but this effect was not statistically significant. In contrast, inoculation of cats with either 10(4) or 10(2) U of rHuIFN-alpha/kg significantly enhanced (P = 0.05 or 0.008, respectively) Con A-induced blastogenesis and had no discernible effect on PWM responses. These findings suggest that very high doses of rHuIFN-alpha given parenterally may be associated with suppression of certain T-cell responses in cats; conversely, much lower doses may be

immunoenhancing.

PMID: 2139993 [PubMed - indexed for MEDLINE]